Fish & Wildlife Division

WILDLIFE CONSERVATION AND BIODIVERSITY SECTION

Testing Methods for Detecting Wolverine

Alberta Species at Risk Report No. 71

Alberta
SUSTAINABLE RESOURCE DEVELOPMENT

104-04273/

Testing Methods for Detecting Wolverine

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Alberta Species at Risk Report No. 71

March 2003

Project Partners:

Alberta
SUSTAINABLE RESOURCE DEVELOPMENT
Fish & Wildlife

Alberta Conservation Association

Publication No.: I/110
ISBN: 0-7785-2829-4 (Printed Edition)
ISBN: 0-7785-2830-8 (On-line Edition)
ISSN: 1496-7219 (Printed Edition)
ISSN: 1496-7146 (Online Edition)

Illustration: Brian Huffman

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This publication may be cited as:

Mowat, G., C. Kyle, and D. Paetkau 2003. Testing methods for detecting wolverine. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Report No. 71. Edmonton, AB. 10 pp.

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ACKNOWLEDGEMENTS

We thank Mike Ewald, Anne Hubbs, and Christine Found for diligent help in the field. Stan Boutin, Rich Moses, and Joe Litke provided useful advice and shared results of their carnivore inventory work. We would like to thank Jeff Copeland, John Krebs, and Eric Lofroth for their considerable input regarding hair sampling for wolverine. We thank Matt Besko and Lisa Wilkinson for their encouragement and support. Kim Poole and Rich Moses reviewed an earlier version of this report. This report was funded by Alberta Environment, NRS, Fisheries and Wildlife Management Division, Resource Status and Assessment Branch, Species at Risk Program.

EXECUTIVE SUMMARY

Mowat (2001) reviewed the methods available for inventory of wolverine (*Gulo gulo*). He concluded that laboratory and field methods needed testing before hair sampling should be used to attempt an estimate of wolverine abundance. To address this concern we tested baited plywood box traps and barbed wire corrals for removing hair from wolverine in late winter. Twelve box traps were set for a total of 622 trap-nights and 1 wolverine was identified at one box trap site on a single occasion. Nine corral traps were set for 474 trap-nights and 1 wolverine was detected 3 times at the same site. Rub pads were set at 14 sites for a total of 677 trap-nights and none detected wolverine. We demonstrated that wolverine hair samples can be taken non-invasively using barbed wire in several different configurations. This result is not unexpected, as both John Krebs and Eric Lofroth have collected wolverine hair samples using baited barbed wire sites and our traps were based on designs suggested by these 2 researchers. Detections (and approaches) were too few to shed any light on which trap type may be the most efficient. Rub pads with catnip and beaver castor bait do not appear to work for wolverine.

We developed a species test based on a 130 base pair fragment of the 16s ribosomal gene. This test was able to separate essentially all species of carnivores occurring in western Canada, as well as many of the rodents, lagomorphs and cervids that are likely to be detected at hair-snagging stations.

We also demonstrate that there is adequate microsatellite variation to identify individual wolverine with relatively small risk of error. Some refinement of the marker suite used for an inventory may still be considered, since a new suite of markers developed specifically for Scandinavian wolverines is now available.

1.0 INTRODUCTION

Mowat (2001) reviewed the methods available for inventory of wolverine (*Gulo gulo*). He concluded that several technical requirements needed testing before hair sampling could be used to produce a reliable estimate of wolverine abundance. He stressed the need to develop an efficient method to remove hair from wolverine in the wild. Estimating capture success (detections per trap-night) for wolverines in several areas of Alberta would aid in study design. An automated species test for boreal carnivores would allow cost-effective and objective species assignment of samples. It would also be useful to select a suite of microsatellite markers that give maximum power to identify individuals using hair samples. In this report we present results from efforts to address the above technical needs based on work done during late winter 2001.

2.0 OBJECTIVES

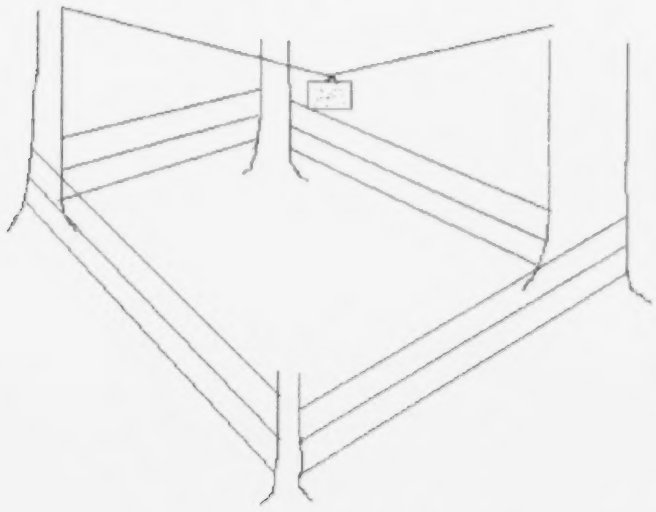
The objectives of this study were to:

1. Test various methods to non-invasively collect hair samples from wolverine.
2. Examine whether contemporary genetic analysis methods could be used to identify wolverine samples of unknown origin to species and individual.

3.0 METHODS

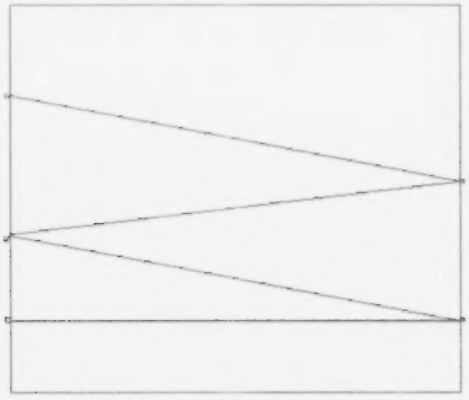
We designed 2 baited hair traps for wolverine based on discussions with wolverine researchers and previous trials using captive wolverine (D. Lewis and D. Fear, unpubl. report). The first trap we used was similar to the barbed wire corral described by Woods et al. (1999) except that we used 3 wires spaced 25 cm from the snow and each spaced 25 cm apart above (Fig. 1). Most sites were baited with rotten beaver meat, which was stored in ammunition boxes that had holes drilled in them. The bait boxes deterred marten, gray jays and other carnivores from depleting the bait and, also assured that wolverine which visited traps were not rewarded; which should alleviate a trap-happy response by some or all individuals. Bait was hung about 2 m above the ground in the middle of the corral on a rope. Lure made of beaver castor, fish oil and fennel and was applied to a tree in the corral. Most corrals were 3-5 m in diameter.

Figure 1. Barbed wire corral showing baited ammunition box suspended from tree on a rope. Three strands of barbed wire are run around each perimeter tree, all separated by 25 cm. Note that holes are drilled in the ammunition box to allow the bait odor to escape. All corrals were >3 m across.



The second trap was a large rectangular 3/4" plywood box (60x60x120 cm long), which was closed at one end. Bait was put in an ammunition box as above, lure was placed in the back of the closed end of the box and, barbed wire was strung across the mouth of the box. The first row of barbed wire was run horizontally 10 cm above the bottom of the box; the next 3 strands were run at angles 15 cm above the previous strand (Fig. 2).

Figure 2. Diagram showing the open end of the baited plywood box and the spacing of the barbed wire, which was attached across the mouth of the box. Each strand of barbed wire was wrapped around wood screws that were drilled into the side of the box 4-6 cm from the end.



In addition we set rub pads baited with beaver castor and oil of catnip at most sites. Rub pads were designed to detected lynx and other Felids, but they have been shown to work on other carnivores such as canids and black bears (McDaniel et al. 2000, Mowat et al. 2000). These pads are 15 cm x 15 cm squares of carpet with roofing nails sticking through the carpet around the edge (McDaniel et al. 2000). Each pad is nailed to a tree at knee height and lure is rubbed into the middle of the pad. Target animals rub their face or shoulder on the lure and leave hair on the nails.

We set 9 wire corral sites and 12 box traps in 3 areas in west central and central Alberta between 7 February and 14 April 2001. Ten sites were set along Hwy. 40 between Hinton and Grande Cache, 5 sites were set along Hwy. 47 between Edson and Robb, and 6 sites were set on Hwy. 881 between Athabasca and Calling Lake. On average each site was active 52 days; sites were checked every 7-14 days.

David Paetkau of Wildlife Genetics International was contracted to design a genetic test that would be able to objectively assign mustelids, and hopefully the other carnivores found in boreal Alberta, to species. Ideally this test would work on small quantities of low quality DNA typically collected using non-invasive sampling, require only a small number of reactions to minimize costs, and be run an on automated sequencer so that a large volume of samples could be handled relatively quickly.

Chris Kyle is studying the genetic structure of wolverine and other mustelids across their North American range (Kyle et al 2000, 2001; Kyle and Strobeck 2001). Chris analyzed

all the wolverine samples collected during our fieldwork and other carcasses, which we collected from trappers and Conservation Officers in the area we worked. Additional carcasses were obtained from Bob McClymont at Alberta Fish and Wildlife, Edmonton. The primers used to amplify the microsatellites were developed by: Davis and Strobeck (1998) in badgers (BA-1 and BA-4), martens (MA-3) and wolverines (GG-3, GG-4, GG-7, GG-14); by Duffy et al. (1998) in wolverines (Ggu 101, Ggu 216 and Ggu 234); by Flemming et al. (1999) in mink (Mvis-75); and by Dallas and Piertney (1998) in Eurasian otters (L-604). Amplification of DNA was performed as in Davis and Strobeck (1998). DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer. Analysis of DNA fragments was done using the programs GeneScan™ Analysis 2.02 and Genotyper® 2.0.

4.0 RESULTS

Wolverine detection rates were very low. Two individual wolverines were detected south of Grande Cache near the Huckleberry and Simonette Look-out Towers. No wolverines were detected Between Edson and Robb or between Athabasca and Calling Lake. Box traps were set for a total of 622 trap-nights and 1 wolverine was identified at one box trap site on a single occasion. Corral traps were set for 474 trap-nights and 1 wolverine was detected 3 times at the same site. Rub pads were set at 14 sites for a total of 677 trap-nights and none detected wolverine; 1 rub pad set near Calling Lake detected a lynx and another detected a white-tailed deer. Box traps detected fisher 4 times at 3 different traps. Corral traps detected wolves 5 times at 2 different traps, coyotes twice at the same trap, and cougar once at a single site.

Several marten and fisher each approached at least 1 box and 1 corral trap, based on tracks in the snow, and were not detected. Marten approached each trap several times. Wolverines approached 1 box and 1 corral trap and were not detected. On 1 occasion, a coyote approached a corral trap without leaving a hair sample.

David Paetkau developed a species test for North American carnivores based on variation in a 130 base pair fragment of the 16s ribosomal gene. This test is somewhat more expensive and labour-intensive to perform than other genetic tests, but has the significant advantage of being able to resolve essentially all species that we have examined. In order to fully test this new genetic marker, as well as to develop a reference database for a wide range of species, we obtained and ran 296 samples of known species from Bob McClymont at Alberta Fish and Wildlife. These samples cover essentially all species of carnivores occurring in western Canada, as well as many of the rodents (including squirrels), lagomorphs and cervids that are likely to be detected at hair-snagging stations. Of all the species that we have examined to date, the only 2 that we are unable to resolve are brown bears and polar bears; the latter being an evolutionarily recent offshoot of the former.

We analyzed the hair samples collected during this work and several other wolverine tissue samples collected from trappers in northwest Alberta at 12 microsatellite loci that were known to work for wolverines (see Kyle and Strobeck 2001). There was more than

adequate variation within these loci to identify individuals (Table 1); several loci could be dropped while still retaining adequate power to assign individuals.

Table 1 presents the results of the microsatellite analysis. The number of alleles found in a population can be used as a measure of genetic variation; the greater the number of alleles the greater the level of genetic variation. It should be noted, however, that this measure can be heavily biased by low sample size, such as in this case. Heterozygosity is less influenced by sample size. This measure represents the percent of individuals that have different alleles at a locus as opposed to having 2 similar alleles at the same locus. P_{ID} is the probability of identity, or the probability that 2 individuals will have the same set of alleles at all loci. The greater the level of genetic variation in a population, the less likely two individuals will have the same alleles. Furthermore, this calculation is multiplicative, so the more loci used, the greater the probability that you have detected an individual and not sampled 2 individuals with the same genotype. In this case, the multiplicative probability of identity is approximately 1 in 671 million. Clearly, there are not 671 million wolverines in this population and so we can be quite confident that we can identify individuals in this sampled region.

Table 1. Genetic variation found at individual microsatellite loci for 20 individual wolverine from the Grande Cache region of Alberta. The heterzygosity is the proportion of individuals that were heterzygote at the given loci in the sample. PID is termed the probability of identity and is the probability that any 2 randomly selected individuals will have the same 2 alleles at the loci in question.

Locus	**Number of Alleles**	**Heterozygosity (%)**	**P_{ID}**
MA-3	5	77	.09
GG-3	3	57	.26
GG-4	5	65	.16
GG-7	6	76	.09
GG-14	6	30	.48
BA-1	5	60	.19
BA-4	4	60	.22
Ggu101b	5	57	.22
Ggu215	6	64	.15
Mvis75	5	68	.16
Ggu234	4	62	.20
Lut604	6	58	.20
Mean/Overall	5.00	61.15	1/671 million

5.0 DISCUSSION

We demonstrated that wolverine hair samples can be taken non-invasively using barbed wire in several different configurations. This result is not unexpected, as both John Krebs and Eric Lofroth have collected wolverine hair samples using baited barbed wire sites and our traps were based on designs suggested by these 2 researchers. Detections (and

approaches) were too few to shed any light on which trap type may be the most efficient. Rub pads with catnip and beaver castor bait do not appear to work for wolverine. Mowat and Stanley (2001) had wolverine approach at least 2 rub pads during a carnivore inventory in southeast British Columbia and on neither occasion was a hair sample collected.

The box type traps detected several fisher while the corral traps did not, despite several approaches. The box trap appears to be a better choice for fisher over the corrals, which makes sense given their small size. Glue based traps would also likely work for fisher and may be preferable because they are simpler and lighter (Foran et al. 1997b). Corral traps also worked for cougar, wolves and coyotes; Poole et al. (2001) also found that corral traps removed hair samples from wolves. The site that repeatedly detected wolf samples was baited with an entire deer carcass.

From a logistic point of view corral traps are cheaper to build and much easier to carry to a site. Trees are necessary because the options for putting up posts in winter are limited. Mixed samples, whether among individuals or species, are less likely with corral traps because the barbed wire is stretched out over a much larger area than in the box traps. Additionally, barbed wire corrals allow the use of large baits such as road-killed ungulates, which may be preferable, especially if sites are not moved.

An objective species test to screen for the species of interest is necessary in the context of a genetic based wolverine inventory because wolverine hair samples can be confused with fisher and wolf based on visual examination alone. Perhaps building a reference collection of hair based on known samples could increase the accuracy of visual sorting. In the short term at least, the species test developed for this project removes the risk of error due to misidentification of the species of a sample, and may generate data on other species of interest such as wolves or fisher.

Alternative species tests are available, such as the one presented by Foran et al. (1997a), but these analyses use restriction enzymes. These tests are cheap to employ and effective, but they require considerable labour. These methods are most suitable in academic settings where labour is relatively inexpensive and capital budgets are often limited. It is generally better to automate as much of the procedure as possible for commercial application to save costs. All genetic tests for grizzly bear inventories use an automated sequencer, which reduces costs and decreases the turn-around time in the lab. It is preferable to develop this capacity for wolverine and other mustelids, such as fisher and otter, because it is likely to reduce costs for large scale inventories. The test developed here can be used to screen samples for the inventory of any carnivore in North America.

We also demonstrate that there is adequate microsatellite variation to identify individual wolverine with relatively small risk of error. Some refinement of the marker suite used for an inventory may still be considered, since a new suite of markers developed specifically for Scandinavian wolverines is now available (Walker et al. 2001). It should also be noted that much of the data in Table 1 was generated using tissue samples. Some

markers do not perform as well with hair samples because of the lower volume of DNA present in the extracted sample. It would also be desirable to use the sibling match probability to assign individual identity of wolverines (as described in Woods et al. 1999), because it is likely we would detect siblings (or parent-offspring) during a large scale inventory.

6.0 MANAGEMENT IMPLICATIONS AND FUTURE DIRECTIONS

We have shown that genetic samples can be collected non-invasively from wolverine, that objective screening for species is possible, and that wolverine in northwest Alberta harbour adequate variation to identify individuals. Performing a broad scale inventory to estimate population size for wolverine in Alberta is theoretically possible. We do not know the capture efficiency of the various hair capture methods and this can only be measured with intensive sampling. We make several suggestions if a population inventory is attempted, many of these are discussed in greater detail in Mowat (2001):

- Use baited corral traps because they are cheaper and logistically more flexible to use.
- Move sites among trapping sessions if a wolverine is detected at a given site in order to minimize behaviour response to a site. Do not move sites which have not yet detected a wolverine; this should save some costs during fieldwork and not affect capture variation.
- Standardize the size and type of bait and lure used during the study; larger baits may maximize capture probabilities and the long-term attraction of a site but are difficult to employ. Protecting baits in containers, such as the ammunition boxes we used, ensures that bait will not be removed while the trap is active. It is acceptable to use different types of bait or lure in different sessions because capture success will likely vary among sessions regardless of the bait used.
- Select an area that is known to have higher (rather than lower) density of wolverines. Results from Petersen (1997) and Poole and Mowat (2001) would suggest the greatest numbers of wolverines occur in 2 areas in the northwest of the province: 1) the area north of the Yellowhead highway between Hinton and Jasper, which includes part of Jasper National Park and the Willmore Wilderness Area, 2) the extreme northwest corner of Alberta north of Peace River.
- Preference should be given to areas that have reasonable accessible during winter to save access costs. The need for helicopter access must be minimized because of the high cost.
- Begin setting sites in early February, check each site every 2 weeks for at least 4 sessions, 6 sessions is preferable which would mean finishing up fieldwork at the end of April.
- Set at least 60 cells in an area of 12,000-15,000 km^2 (Mowat 2001).

From a conservation point of view, an inventory should generate an estimate of abundance that could be used to estimate density. This parameter combined with available estimates of survival and recruitment could be used to predict whether current harvest rates are sustainable. The error in the recording of wolverine harvest is likely to be large with the current harvest tracking system (Poole and Mowat 2001) because many

wolverine pelts go into local (or semi-local) parka industries, which means they do not reach commercial auction houses. These pelts can only be recorded in the volunteer harvest submission; often trappers do not bother to record harvests for species they do not send to auction because they must then pay a royalty for the pelt, which they normally avoid in a local sale. This error warrants further investigation and may require modifications to the tracking system.

Alternatively, track counts could be a cost-effective method to detect and monitor wolverine distribution (Zielinski and Kucera 1995). Population estimates are not possible with track counts but they can be used to measure relative abundance. Track counts can be difficult to standardize and carry out due to variation in snow conditions and animal movement patterns among areas. And, wolverine tracks can be confused with several more abundant species such as lynx and wolves, especially in deep, soft snow. Some of this variation can be overcome with observer training, a standardized sampling design, and repetition of counts. Track counts are cheap compared to a genetic inventory as long as a helicopter is not needed for access. Large areas can be surveyed and a monitoring design has been proposed (Moses et al. 2001), and field-testing of the monitoring protocol has begun in eastcentral Alberta (Richard Moses and Stan Boutin, personal communication).

Detection surveys allow the comparison of abundance among areas if sampling intensity is adequate. Because detections will be few for wolverine and they have large home ranges, comparisons must be restricted to large areas. Many sample sites will not detect a wolverine due to their low abundance so tens of sites would need to be measured in each area. Detection data can also be used to monitor population trend and changes in distribution across time (see Poole and Mowat [2001] for a simple example of this kind of analysis of distribution). Long-term data across very broad scales are necessary for this type of analysis and variations in capture success among surveys may make detection of population trends difficult. The analysis of distribution, i.e. an analysis to detect a decline in distribution of a population, may be the most efficient way to monitor populations across large jurisdictions like a province over time.

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